



Sexually selected signal attracted females before deterring aggression in rival males

MOLLY R. MORRIS, M. SCARLETT TUDOR & NATALIE S. DUBOIS

Department of Biological Sciences, Ohio University

(Received 27 October 2006; initial acceptance 8 December 2006;
final acceptance 15 January 2007; published online 23 October 2007; MS. number: A10604)

Many sexually selected male signals elicit responses in males and females and, as a result, are affected by selection due to both mate choice and competition for mates. It has been proposed that because signals used in aggressive interactions may also be reliable indicators of mate quality, these dual functioning traits initially evolved in the context of male–male competition and were later co-opted by females to assess potential mates. Our phylogenetic reconstruction of the ancestral states of male and female responses to the sexually selected trait ‘vertical bars’ in the northern swordtail fish, *Xiphophorus* spp., suggests that female response to the bars was present before male response to the bars evolved. This is the first evidence that males may have co-opted a signal for use in male competition that was initially used by females in mate choice. We suggest that dual functioning, sexually selected traits are as likely to initially evolve in the context of female preference as in the context of male–male competition. In addition, we propose that eavesdropping may be an important mechanism by which signals are co-opted, with behavioural syndromes potentially playing a key role in the transition of the signal from a context of courtship to one of aggression.

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Keywords: ancestral reconstruction; behavioural syndrome; eavesdropping; female mate preference; male–male competition; phylogenetic analysis; sexual selection; sexual signal; swordtail fish; *Xiphophorus*

The two components of sexual selection, intra- and intersexual selection, have been characterized as selecting for two different types of traits: weapons or armaments via male–male competition, and ornaments via female mate choice (Darwin 1871). However, many sexually selected male traits are affected by both intra- and intersexual selection (Tinbergen 1953; Ord et al. 2001) and often function as signals with dual audiences: females assessing males as potential mates and males assessing males as competitors (Andersson 1994). For example, the sword, a classic sexually selected male ornament preferred by females in *Xiphophorus helleri* (Basolo 1990), plays a role in male–male competition as well (Benson & Basolo 2005). The discovery that ornamental traits may be used as indicators of competitive ability in male–male competition has led to the proposal that secondary sexual traits with dual functions originated in the context of male–male

competition and were later co-opted by females for mate preference (Borgia 1979; Berglund et al. 1996). This hypothesis is based on the assumption that signals used in aggressive interactions are more reliable indicators of male quality, as dishonest signals can lead to immediate and negative consequences for a male in the context of male–male competition but may have less immediate consequences in the context of female mate preference (Berglund et al. 1996). One study has provided phylogenetic support for the ‘war propaganda’ (Borgia 1979) or ‘armaments–ornaments’ (Berglund et al. 1996) hypotheses: in bowerbirds, the ‘skraa’ call evolved as an aggressive signal before its use as a courtship signal (Borgia & Coleman 2000).

One mechanism by which signals could be co-opted from one context of sexual selection to another is eavesdropping. Eavesdropping occurs when a receiver extracts information from a signalling interaction in which it has taken no part (McGregor 1993). Communication network theory suggests that signals are often available to many different individuals in the community, not just the

Correspondence: M. R. Morris, Department of Biological Sciences, Ohio University, Athens, OH 45701, U.S.A. (email: morrism@ohio.edu).

intended audience (McGregor & Peake 2000). For example, females have been shown to use information gained via eavesdropping on male contests in subsequent mate choice decisions (Doutrelant & McGregor 2000). Thus, if eavesdropping on male–male contests allows females to differentiate among males in a way that results in higher net fitness for females (Wong & Candolin 2005), this might eventually lead to co-option of intrasexual signals into an intersexual context. But, when rival males and potential mates are interested in the same information about a male, the reverse might also be true. If the boldness that a male shows while courting is correlated to the level of aggression he will show in the context of male–male competition (a behavioural syndrome, Gosling 2001), males that eavesdropped on courtship signals could gain useful information about potential competitors.

Determining the context in which traits that function in both female mate choice and male–male competition initially evolved has implications for the evolution of sexually selected signals as well as female mate preferences. First, signal design is strongly influenced by the receiver (Endler 1992), and therefore aspects of current signal design could depend on whether the initial receiver was a male competitor or a potential mate. Second, showing that a sexually selected trait initially evolved as an aggressive signal and was later co-opted by females for use in mate choice would rule out some of the theoretical models for the evolution of female mate preference for that trait (see Berglund et al. 1996). Finally, determining the context in which dual functioning, sexually selected traits initially evolved could suggest the extent to which we would expect males to eavesdrop on signals used in courtship interactions as compared to females eavesdropping on signals used in male–male interactions.

'Vertical bars' is a pigment pattern (Gordon 1931; Atz 1962; Fig. 1) found throughout swordtails and platyfish (*Xiphophorus*), and is known to function as a sexually selected signal that both deters rival males and attracts females in some species (Morris et al. 1995; Morris & Ryan 1996). In those species of northern swordtail fish with this pigment pattern, males express their bars when

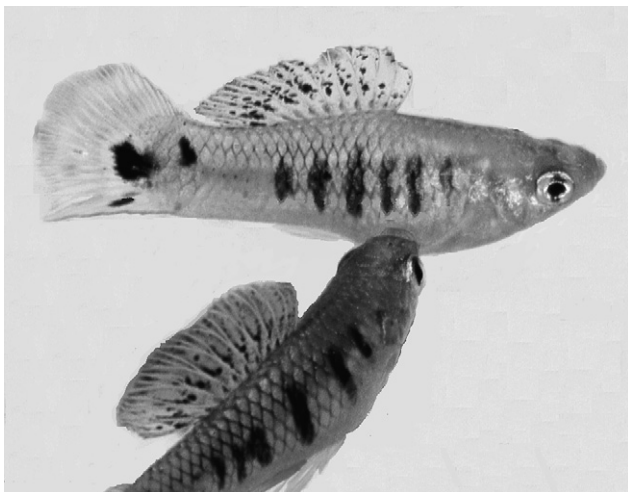


Figure 1. *Xiphophorus cortezi* males with vertical body bars expressed during male–male interaction.

courting females and during aggressive encounters with rival males and suppress their bars when they lose an aggressive encounter (Morris et al. 1995; Moretz & Morris 2006). Determining the context in which a sexually selected trait initially evolved requires a phylogenetic analysis. Therefore, we determined the ancestral states of both male and female responses to the sexually selected signal 'vertical bars' for the northern swordtail clade of fish so that we could assess whether this signal initially evolved in the context of male–male competition (male response evolved first), female mate preference (female response evolved first), or both (evolved at same time).

METHODS

Study Species

A previous study determined male response to the bars in all of the northern swordtail species (Moretz & Morris 2006), and female preference for the bars (hereafter referred to as female response) has been examined in many of the northern swordtail species (Morris et al. 1995, 2003, 2005; Morris & Ryan 1996; Morris 1998). In this study, we measured female responses to the bars for the remaining untested northern swordtail species (*Xiphophorus nezahualcoyotl*, *Xiphophorus montezumae*, *Xiphophorus birchmanni* and *Xiphophorus malinche*), and a species of platyfish as an outgroup taxon (*Xiphophorus variatus*) using dichotomous choice tests. In addition, we tested more than one population for two species that occur in hybrid zones (*X. malinche* from the Rio Calnali High, and *X. birchmanni* from the Rio Xilitatl) and we retested one species (*Xiphophorus pygmaeus*) to compare our results using video animations as stimuli with those of previous studies that used live males as stimuli. All females were collected as adults in the field and isolated into individual 20-litre aquaria at least 1 week before testing.

Female and Male Responses

We tested the responses of females using the same video animations (one with a symmetrical number of bars and one barless) and methods used by Morris et al. (2003). The time that the females spent in the third of the aquarium closest to an animation was recorded across two trials for each test, switching the side on which the animations were presented between trials to control for side biases, and testing each female twice to examine consistency. The total time that a female spent in the compartment adjacent to each animation across trials was measured, and the difference in the total time spent with each animation was used as a measure of the strength of response. Time associating with a stimulus is a good measure of mating preference in two other fish species: in the closely related swordtail *Xiphophorus nigrensis* (Ryan et al. 1990; Morris et al. 1992) as well as in another live-bearing fish in which males use mating tactics that are similar to those of *X. pygmaeus* (Bizazza et al. 2000).

Methods for measuring male response, as well as the results for all northern swordtail species and *X. variatus*

can be found in [Moretz & Morris \(2006\)](#). Briefly, the difference in the number of bites that a male directed towards his mirror image with bars versus without bars was used as a measure of response to vertical bars. To manipulate the presence and absence of vertical bars, bars were removed using dry ice and added using Dr. Blue Kote dye (H. W. Naylor Co., Inc., Morris, NY, U.S.A.). In addition, for those species with a polymorphism for vertical bars, the two morphs were analysed separately.

Statistical Analyses

We determined whether there were any differences in the overall time spent associating with the video animations across species with a one-way ANOVA. Wilcoxon matched pairs signed-rank tests were used to test whether there was a significant response to bars for each species. In cases in which there was a significant response to the bars, statistics for the first test are presented. If no significant response was detected, we examined the variation in strength of response across two tests with a one-way ANOVA. Populations that showed significantly greater variation across females as compared to within a female, with some females spending more time with the animation with bars and some with the animation without bars, were scored as polymorphic. If both the Wilcoxon matched-pairs signed-ranks test and the one-way ANOVA were not significant, the population was scored as having 'no response'. The strength of response for species scored as having 'no response' was compared to the outgroup taxon using a Mann–Whitney *U* test.

Ancestral State Reconstruction

We scored the responses of males and females to the bars in two ways: (1) COMPLEX = different types of responses were scored as different states of the response; (2) SIMPLE = differences in types of responses, as long as a response could be detected, were all scored as response 'present'. The COMPLEX score for female response had four states: 0 = no response, 1 = more time with bars, 2 = more time with barless, 3 = polymorphism with some females spending more time with barred animation, some spending more time with barless animation. The SIMPLE score for female response had two character states: 0 = no significant response, 1 = significant response

(either 'bars', 'barless', or 'polymorphic'). The COMPLEX score for male response had four states: 0 = no response, 1 = more aggression towards bars, 2 = decrease aggression towards bars, 3 = polymorphism with some males responding with decreased aggression towards bars, some having no response. The SIMPLE score for male response had two character states: 0 = no response, 1 = significant response (either 'increased bars', 'decreased bars', or 'polymorphic'). We reconstructed the ancestral states for both the signal and response using maximum parsimony as implemented in MacClade ([Maddison & Maddison 2002](#)) and maximum likelihood as implemented in Mesquite ([Maddison & Maddison 2005](#)).

The maximum likelihood analyses used the MK1 model of evolution, which assumes an equal rate of change between all states for the responses of both sexes. Character states were ordered in the maximum parsimony analysis of the COMPLEX scorings for both sexes ([Table 1](#)). For female responses, we assigned one step to change from no response to a response for bars, a response for barless, or the polymorphic state. We assigned two steps for a change from response for bars to a response for barless, as this change would require either a loss of one response and the gain of the other, or one gain to change to the polymorphic state followed by one loss ([Table 1](#)). We ordered the character states for male responses based on the following two models. In both models, we assumed that populations with or without the response go through the stage of being polymorphic for response before losing or fixing the response. In the first model (more steps), we assigned the evolutionary change from one response state to another (male responses of increased aggression towards bars and decreased aggression towards bars) four steps: first a change to polymorphism (response/no response), then to absent, and then another polymorphism before becoming fixed in the other state ([Table 1](#)). In the second model (fewer steps) we allowed a direct transition between the two types of responses. The second model assumes that once a response to the bars has evolved, it is not difficult to shift the context in which the response is given ([Table 1](#)).

The phylogenetic tree we used for our analysis is supported by data from several different sources and data types, including morphological ([Rosen & Kallman 1969](#); [Rauchenberger et al. 1990](#)), behavioural ([Heinrich & Schröder 1986](#); [Haas 1992](#)), geographical ([Rosen 1978](#), [1979](#)), protein electrophoretic ([Rauchenberger et al.](#)

Table 1. Matrices used to order COMPLEX character states for parsimony analyses

Female					Male (more steps)					Male (fewer steps)				
State	0	1	2	3	State	0	1	2	3	State	0	1	2	3
0	0	1	1	1	0	0	2	2	1	0	0	2	2	1
1	1	0	2	1	1	2	0	4	3	1	2	0	1	2
2	1	2	0	1	2	2	4	0	1	2	2	1	0	1
3	1	1	1	0	3	1	3	1	0	3	1	2	1	0

Number of steps (gains or losses) to change from one state to another are indicated. Character states for female responses: 0 = no response, 1 = more time with bars, 2 = more time with barless, 3 = polymorphism between states 1 and 2. Character states for male responses for both 'more steps' and 'fewer steps' models: 0 = no response, 1 = increased aggression towards bars, 2 = decreased aggression towards bars, 3 = polymorphism between states 0 and 2.

1990; Chen 1993; Morris et al. 2001a) and restriction fragment length polymorphism (Schartl 1990; Borowsky et al. 1995). However, there is some evidence to question the placement of two species within this clade (Morris et al. 2001a; Gutiérrez-Rodríguez et al. 2007) and therefore we conducted a subset of the analyses on two alternative topologies: (1) placing *X. continens* as the sister species of *X. montezumae*; and (2) placing *X. malinche* as the sister species of *X. cortezi*. Branch lengths used in the maximum likelihood analyses were calculated from cytochrome *b* sequences (L. Dries, unpublished data) using PAUP* v.4.0, beta 10 (Swofford 2002). Starting trees were obtained via stepwise addition for tree-bisection-reconnection (TBR) branch swapping, saving in memory one tree at each step during stepwise addition, and keeping only those trees compatible with the Rauchenberger et al. (1990) phylogeny. Hierarchical likelihood ratio tests identified the evolutionary model HKY85 + G + I as most appropriate for the data, with the following initial parameter estimates as the appropriate model: transition/transversion (Ti/Tv) ratio = 7.474, proportion of invariable sites (*i*) = 0.4480, alpha shape parameter (α) = 0.57284, and base frequencies $A = 0.2760$, $C = 0.3244$, $G = 0.1395$, $T = 0.2600$. To determine the robustness of our results based on analyses using branch lengths calculated from mtDNA, we also conducted a subset of the maximum likelihood analyses using equal and Pagel branch lengths (Pagel 1994). The Pagel branch lengths were implemented in Mesquite with the PDTREE module (Garland & Diaz-Uriarte 1999).

RESULTS

There was no significant difference between species in the time that females spent associating with the video animations (ANOVA: $F_{7,75} = 1.88$, $P = 0.084$), which suggests that the animations elicited similar levels of response across species. *Xiphophorus nezahualcoyotl* and *X. variatus* females from two different populations spent more time associating with the barred as compared to

the barless video animation (Table 2). *Xiphophorus pygmaeus* females spent significantly more time associating with the barless video (Table 2), the same response that was detected for this species when using live males as stimuli (Hankison & Morris 2002). *Xiphophorus malinche* females from both populations, on the other hand, had no significant response to the bars in either test (Table 2). However, variation in strength of response to the bars was greater across females than within females for both populations of *X. malinche* (Calnali: $F_{10,11} = 5.12$, $P < 0.01$; Rio Claro: $F_{8,9} = 5.5$, $P < 0.01$; Fig. 2a), indicating a polymorphism in female response for bars (Morris et al. 2001b). The response to the bars by *X. montezumae* females was similar to that of *X. malinche* females. Females did not have a significant response to the bars in either test (Table 2), however, there was significant variation across females ($F_{10,11} = 8.36$, $P < 0.02$; Fig. 2b), with some females spending more time with the barred animation and some spending more time with the barless animation. Therefore, *X. malinche* and *X. montezumae* were both scored as being polymorphic for female response to the bars. Finally, we detected no significant difference in the time that *X. birchmanni* females spent with the barred animation as compared to the unbarred animation for either population in either test (Table 2), nor was there a significant difference in the variation across females as compared to within females for either population (Rio Xiliatl: $F_{10,11} = 1.10$, $P = 0.44$; Rio Garces: $F_{13,14} = 1.1$, $P = 0.43$; Fig. 2c), suggesting that *X. birchmanni* females have either a weak preference or no preference for vertical bars. We found a significantly weaker strength of response for bars in *X. birchmanni* from Rio Xiliatl compared to the strength of response for bars detected in *X. variatus* ($Z = 2.3$, $N_1 = N_2 = 18$, $P < 0.02$). Therefore, while we cannot rule out the possibility that females of *X. birchmanni* have a response to the bars, we scored *X. birchmanni* as 'no response' because their average strength of response was weaker than the response for bars detected in the outgroup taxon.

Table 2. Female response to bars, measured as time associating (s) with barred as compared to time spent with barless video animation, for species tested in current study

Species	Collection site	Test	Time bars (SD)	Time barless (SD)	Wilcoxon signed-ranks test		
					Z	N	P
<i>X. variatus</i>	Rio Garces	1	616.9 (274.82)	369.8 (275.87)	-2.03	7	0.04
	Rio Xiliatl	1	729.3 (232.26)	194.3 (247.73)	-2.77	21	0.005
<i>X. montezumae</i>	Tributary to Rio Gallinas	1	399.9 (383.08)	557 (371.64)	0.71	11	0.48
		2	455.3 (298.95)	528.9 (312.05)	0.36	11	0.72
<i>X. nezahualcoyotl</i>	Tributary to Rio El Salto	1	492.4 (210.54)	360.9 (193.77)	-2.23	14	0.03
<i>X. malinche</i>	Rio Claro	1	515.9 (274.82)	633.8 (275.87)	0.89	9	0.37
		2	450.2 (228.33)	434.5 (249.05)	0.18	9	0.96
	Calnali (high)	1	397.9 (280.17)	455.9 (272.94)	0.62	11	0.53
		2	455.9 (272.94)	463.9 (146.07)	0.18	11	0.89
<i>X. birchmanni</i>	Rio Xiliatl (pumphouse)	1	359.8 (204.9)	426.5 (264.8)	0.36	11	0.72
		2	381.5 (193.32)	322.1 (231.4)	0.36	11	0.72
	Rio Garces	1	346.1 (213.6)	530.6 (195.9)	1.16	14	0.25
		2	465.6 (172.5)	320.1 (167.5)	-1.73	14	0.08
<i>X. pygmaeus</i>	Rio Huichihuayan	1	219.1 (232.26)	588.9 (247.73)	2.03	7	0.04

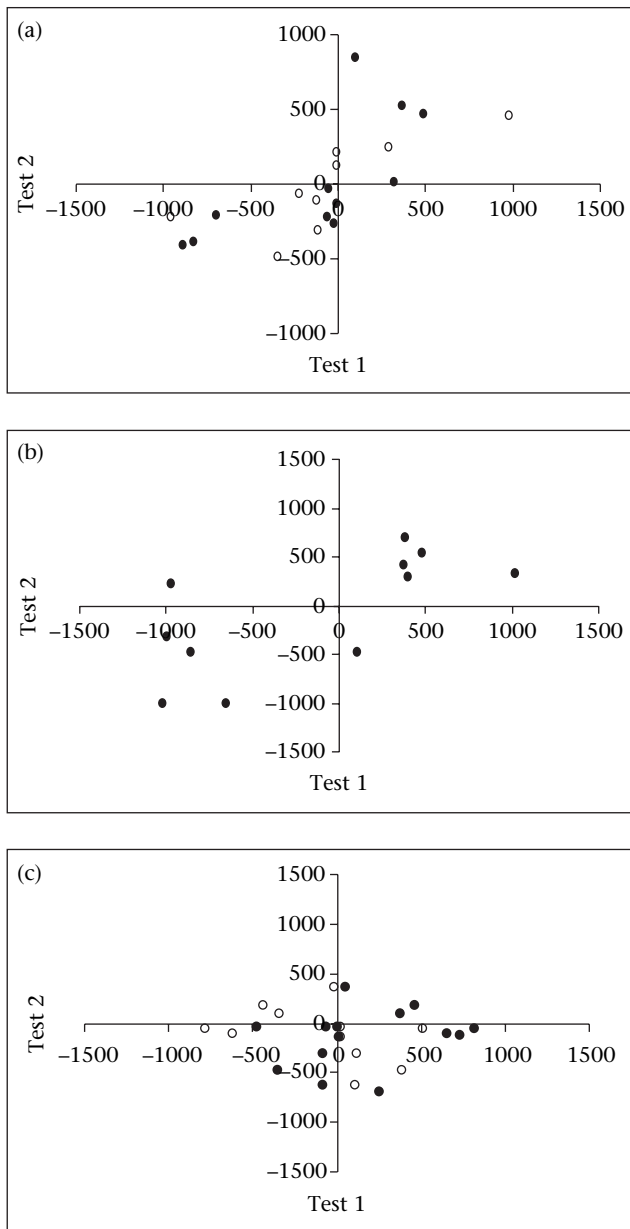


Figure 2. Comparison of the strength of preference (time with barred animation – time with unbarred animation, in seconds) across two tests for the three species that did not have a significant response on average. (a) *X. malinche*, open circles = Calnali, closed circles = Rio Claro; (b) *X. montezumae*; and (c) *X. birchmanni*, open circles = Rio Xilitl, closed circles = Rio Garces. *X. malinche* and *X. montezumae* were scored as ‘polymorphic’, while *X. birchmanni* was scored as ‘no response’.

Table 3 summarizes scoring of the responses of both males and females to the bars for all nine species of northern swordtails and the two populations of *X. variatus*. When both male and female responses were scored using the COMPLEX scoring scheme (four character states), both maximum parsimony and maximum likelihood reconstructed the ancestral states such that male response evolved after female response (Fig. 3). The SIMPLE scoring scheme (only two character states for the responses of both sexes, present or absent) analysed using both

maximum parsimony and maximum likelihood also indicated that female response was present when male response evolved. However, for male response, only the node at the base of the northern swordtails was statistically significant (node 2), while for female response, all of the internal nodes (nodes 2–6) were significant. These results were robust to the two models (more steps and fewer steps) used to order all four character states for male response in the maximum parsimony analysis in the COMPLEX scoring scheme, as well as the analyses based on two additional tree topologies (results not shown). For male response, the results were the same for all three tree topologies, while for female preference, the node at the base of the *montezumae* clade (node 4) was no longer significant in the maximum likelihood analysis when *X. continens* was considered as the sister taxon to *X. montezumae*. However, when *X. malinche* was considered as the sister taxon to *X. cortezi*, the ancestral reconstruction of female preference at the node at the base of the *cortezi* clade (node 5) was significant. The results were also robust to the branch lengths used in the maximum likelihood analyses. Given the SIMPLE scoring for both characters, the Rauchenberger et al. (1990) phylogeny and equal branch lengths, the ancestral state for female preference was statistically significant for ‘response present’ to bars, and for male response, it was statistically significant for ‘no response’ to bars at nodes 1, 2 and 4 (Table 4). The results were similar, given Pagel branch lengths, except that only node 4 was statistically significant for both responses (Table 4).

DISCUSSION

Female response to the sexually selected trait vertical bars was present when male response evolved in the northern swordtail fish, suggesting that males co-opted a signal for use in male competition that was initially used by females in mate choice. We suggest that sexually selected traits are as likely to initially evolve in the context of female preference as in the context of male–male competition. So far, only one other study has examined this question in a phylogenetic context, and the results from that study supported the war propaganda or armament–ornament hypotheses (Borgia & Coleman 2000). While Borgia & Coleman’s (2000) study provides an example of females co-opting a signal that initially evolved in the context of male–male competition, it is not clear that the information from signals used in male–male competition will always be the information that females are seeking when making mate choice decisions. Females do not always prefer dominant males, and dominance does not always predict high-quality males (for review see Qvarnström & Forsgren 1998). In addition, the argument that signals used in aggressive interactions may be more reliable indicators of mate quality, if trait honesty were more easily maintained under male–male competition, ignores selection on the receiver, in this case females in the context of courtship, which is also important in the evolution of communication systems. Direct selection on females due to their mating preferences could drive females to ignore unreliable male signals. Instead, the pattern of signal

Table 3. Presence of vertical bars, collection sites, and character scoring for the responses of both sexes to bars

Species	Bars on males	Collection site	Female responses		Male responses	
			N	Score	N	Score
<i>X. variatus</i> outgroup	Yes	La Cienega	7 (V)	Bars	10 ⁶	NR
		Rio Xiliatl	21 (V)	Bars		
<i>X. montezumae</i>	Polymorphic	Tributary to Rio Gallinas	11 (V)	Polymorphic	14 ⁶	NR
<i>X. nezahualcoyotl</i>	Yes	Tributary to Rio El Salto	14 (V)	Bars	12 ⁶	NR
<i>X. continens</i>	No	Rio Ojo Frio	11 (V) ¹	Polymorphic	12 ⁶	NR
<i>X. malinche</i>	Polymorphic	Rio Claro	9(V)	Polymorphic	11 ⁶	Increased aggression
		Calnali (high)	11 (V)	Polymorphic		
<i>X. birchmanni</i>	Yes	Rio Xiliatl (pumphouse)	11 (V)	NR	13 ⁷	Increased aggression
		Rio Garces	14 (V)	NR		
		Arroyo La Conchita	10 (L) ²	Polymorphic		
<i>X. cortezi</i>	Polymorphic	Arroyo La Conchita	10 (V) ²	Polymorphic	17 ⁷	Polymorphic (decreased aggression/NR)
		Nacimiento Rio Choy	8 (L) ³	Bars		
<i>X. nigrensis</i>	No	Rio Coy	12 (L) ³	Bars	12 ⁷	NR
<i>X. multilineatus</i>	Yes	Rio Huichihuayan	15 (L, cortezi) ⁴	Barless	9 ⁶	NR
<i>X. pygmaeus</i>	No	Rio Huichihuayan	6 (L, multi) ⁵	Barless		
		Rio Huichihuayan	7 (V)	Barless		

Female response was tested with either live males (L) or video animations as stimuli (V), polymorphic = bars and barless. NR = no response. Numbers in superscripts indicate the source of data published elsewhere: (1) Morris et al. (2005); (2) Morris et al. (2003); (3) Morris & Ryan (1996); (4) Hankison & Morris (2002); (5) Morris et al. (1995); (6) Moretz & Morris (2006); (7) Moretz & Morris (2003).

evolution that we detected suggests that sexually selected signals can initially evolve in the context of female mate choice with males co-opting these signals as reliable indicators of aggressive behaviour.

A potential mechanism for the co-opting of a signal that initially functioned in female mate choice for use in male–male competition is eavesdropping. Eavesdropping occurs when a receiver extracts information from a signalling interaction in which it has taken no part (McGregor 1993). In fish, many studies have focused on the information available in aggressive interactions between males (reviewed in Peake & McGregor 2004). However, eavesdropping need not be limited to intrasexual interactions. Eavesdropping on intersexual signals exchanged during courtship has been observed in the context of alternative mating tactics. For example, male European robins, *Erithacus rubecula*, may eavesdrop on within-pair ‘seep’ calls to locate extrapair mates (Tobias & Seddon 2002), whereas sneaker males in the peacock blenny, *Salarias pavo*, choose males to parasitize using independent cues that are used by females to select mates as well as social cues in the form of intersexual copying (Gonçalves et al. 2003). This latter example might represent the co-option of a trait used in female choice into an intrasexual context. The results of our study suggest that eavesdropping on intersexual signals may also occur in the context of males eavesdropping on courtship interactions to gain information about future competitors. Therefore, the mechanism of co-opting a signal via eavesdropping could occur regardless of the context in which the signal initially evolved.

Within the northern swordtails, males have the ability to rapidly intensify as well as rapidly suppress the expression of the bars (Franck 1964; Zimmerman & Kallman 1988; Moretz & Morris 2003). Male response to vertical bars evolved when the timing of the expression of the bars (turning the bars off and on) became an honest signal of aggressive intent (males always turn on bars

before biting, Moretz & Morris 2006). We suggest a scenario in which males may have initially obtained information about competitors from their use of vertical bars as a courtship signal. Eavesdropping on courtship interactions would have been beneficial to males if there was a correlation between how bold a male behaved during courtship (e.g. more likely to keep this highly detectable courtship signal ‘on’ even in the presence of a predator) and his propensity to be aggressive during male–male competition. This relationship seems reasonable given that correlations between antipredator behaviour and aggression first detected in stickleback fish (Huntingford 1976) are now known for several different organisms (Sih et al. 2004). It would be worth examining the possibility of a new role for behavioural syndromes (i.e. correlations between individual behaviours in different functional contexts, Gosling 2001), as part of the mechanism by which sexually selected traits are co-opted from one function to another.

Our study does not allow us to determine when female response to vertical bars, or mate preference for bars, initially evolved. Females from the outgroup (*X. variatus*) preferred vertical bars, and reconstructions of the ancestral state of female response suggest that preference was present at the base of the northern swordtails. Ongoing studies of female preference for bars in the southern swordtails and platyfish may be able to provide insight into this question. Of particular interest, however, is evidence that the bars are not used in courtship in *X. helleri* (a southern swordtail), but instead are expressed in situations in which males are stressed (Basolo & Delaney 2001; A. L. Basolo, personal communication). While further studies are necessary to determine the context in which vertical bars initially evolved, this observation (i.e. bars not expressed during courtship) suggests that the ability to vary the expression of the bars may have evolved before their use as a signal in female mate choice.

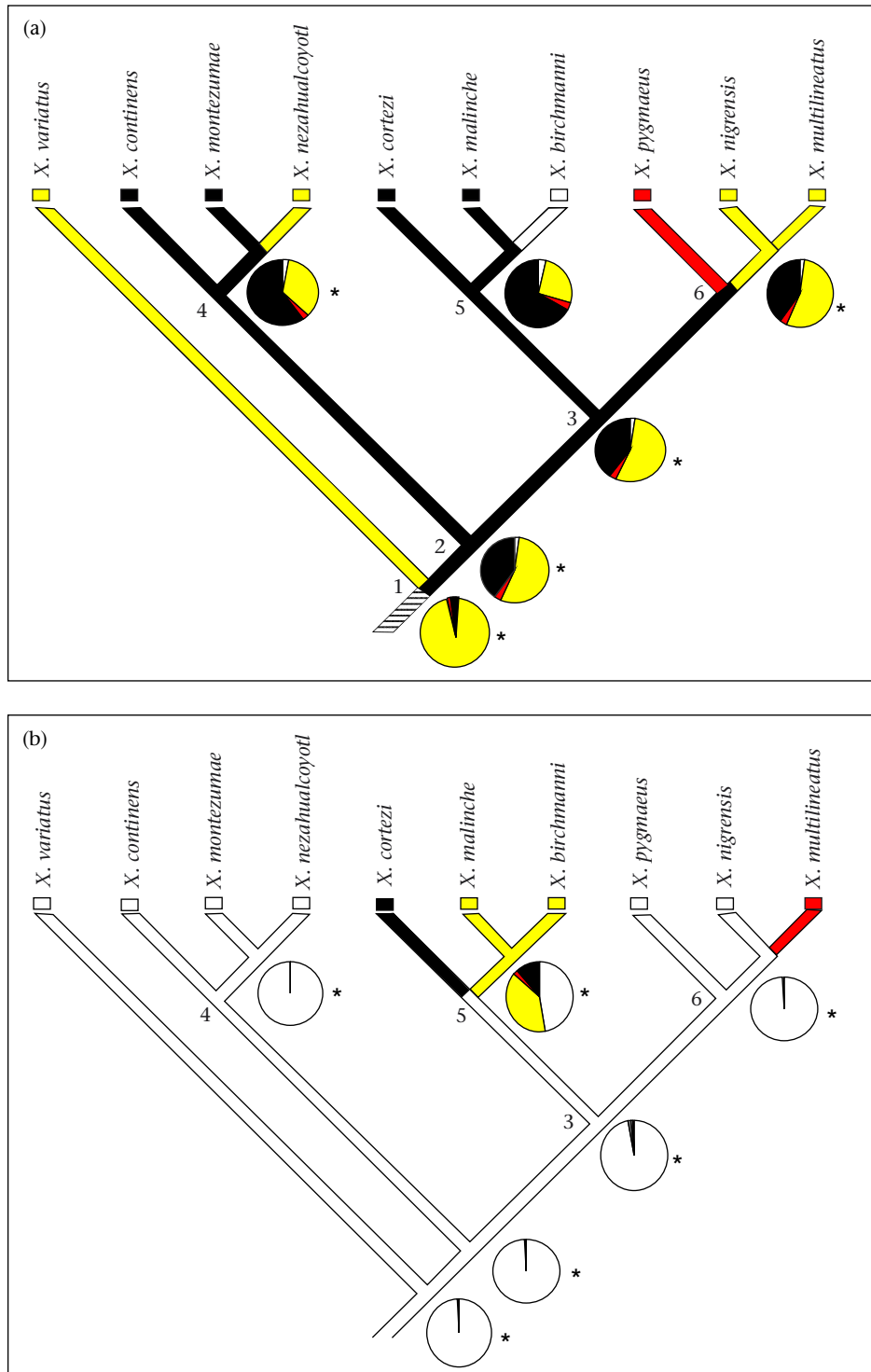


Figure 3. Reconstruction of ancestral states using maximum parsimony and maximum likelihood for the COMPLEX character scorings using the Rauchenberger et al. (1990) phylogeny. Branches are coloured based on maximum parsimony reconstruction. Areas of pies indicate relative support for the different ancestor states using maximum likelihood. Likelihood decision threshold was $T = 2$. Asterisk next to pie indicates one or more significant states. (a) Ancestral states for female preference for bars: no preference (white), preference for bars (yellow), preference for no bars (red), polymorphism (black), equivocal between polymorphism and preference for bars (hatched). (b) Ancestral states for male response to bars: no response (white), increased aggression towards bars (yellow), decreased aggression towards bars (red), polymorphism of no response and decreased aggression towards bars (black), equivocal (hatched).

Table 4. Maximum likelihood ratios for male and female responses scored SIMPLE, using alternative branch lengths

Node	Female		Male	
	No response	Response present	No response	Response present
Equal branch lengths				
1	0.00440	0.99560	0.90989	0.09011
2	0.00006	0.99937	0.88776	0.11224
3	6.34E-05	0.99937	0.68876	0.31124
4	3.20E-06	0.99968	0.97015	0.02985
5	0.00440	0.99560	0.13508	0.86492
6	3.20E-05	0.99968	0.81801	0.18199
Pagel branch lengths				
1	0.01129	0.98871	0.60565	0.39435
2	0.00134	0.99865	0.61354	0.38646
3	0.00005	0.99951	0.48083	0.51917
4	0.00005	0.99954	0.89467	0.10533
5	0.00448	0.99552	0.17091	0.82909
6	0.00002	0.99976	0.62010	0.37999

Nodes correspond to those marked on the phylogeny in Fig. 3. Likelihood decision threshold was $T = 2$ (indicating at least 7.4 times greater support than for another state, Schluter et al. 1997). If a 'best state' by this criterion could be identified, it is highlighted in bold and is considered significant.

This hypothesis does not rule out the possibility that expression of the bars was assessed by females as a cue, providing females with information about whether males were stressed in situations other than courtship, only that expression of the bars did not initially evolve to attract females.

It is possible that the example we present here is not common, and that most sexually selected male traits do initially evolve in the context of male–male competition. However, our results clearly indicate that dual functioning signals can be present as signals in female mate choice before their function as signals in male–male competition. Ultimately, more studies that incorporate phylogenetic analyses are necessary to determine the context in which exaggerated male traits have evolved. In addition, we suggest that eavesdropping by males on courtship signals should be examined as a potential mechanism for the co-opting of signals from the context of courtship to male–male competition.

Acknowledgments

We thank Kevin de Queiroz, André Fernandez and Jason Moretz for comments on the manuscript, Geoff Baker, Abby Darrah, Jason Moretz, Carla Gutiérrez-Rodríguez and Oscar Ríos Cárdenas for assistance in the field, Kevin de Queiroz for the photograph of the *X. cortezi* males, the Mexican Government for giving permission to collect the fish, and NSF (IBN 9983561) as well as Ohio University (Research Incentive) for funds that supported this research. All experiments comply with current laws of the United States and with the Animal Care Guidelines of Ohio University (Animal Care and Use approval no. L01-01).

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